Studies in the Calcium and Phosphorus Metabolism of the Crab, *Podophthalmus vigil* (Fabricius)[1]

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ABSTRACT: By employing modifications of the molt classification by Drach (1939) and Hiatt (1948), it was discovered in laboratory-maintained crabs (*Podophthalmus vigil*) that a partial desiccation occurred during proecdysis followed by a rehydration at the A stages.

The inorganic and organic content of the carapace, mid-gut gland, gills, and muscles were followed during the molt cycle. The carapace had the greatest inorganic fluctuations. The mid-gut gland and muscle tended to increase in both organic and inorganic matter during premolt, suggesting that these organs may serve as reservoirs for these components.

The calcium and total phosphorus constituents of these organs and of the blood were determined at the various molt stages. Fluctuations in the amounts of these two elements were observed in all sampled tissues. The storage of calcium in the mid-gut gland and muscles during premolt is discussed. Phosphorus was found to be stored in the digestive gland during postecdysis but not in proecdysis. The muscle also tended to store phosphorus during premolt.

As *P. vigil* becomes older, i.e., larger, it is unable to resorb from the exoskeleton the same quantity of calcium, but it is able to recalcify the new exoskeleton to the same extent as does a smaller crab.

CALCIFICATION and hard tissue formation occurs in many forms of life. It is found in bacteria (Ennever, 1963; Rizzo, et al., 1963; Greenfield, 1963), algae (e.g., *Porolithon* and *Halamita*), protozoans (Isenberg, et al., 1963; Bé and Ericson, 1963), coelenterates, echinoderms, molluscs, arthropods, and vertebrates. Generally, the function of calcification is to give form, support, and protection, and to contribute in ionic homeostasis (Urist, 1962), but in some instances calcification can be considered a pathological condition. The calcium complex deposited may be in three forms—calcite, aragonite, and apatite. The latter is a calcium phosphate [$Ca_{10}(PO_4)_6(OH)_2$] and the others are calcium carbonate complexes. Very little phosphorus is found in calcite and aragonite, which are generally restricted to the lower phyla. The amount of strontium and magnesium, the crystal structure, and the density of the calcium carbonate determine the difference between aragonite and calcite. The latter has little strontium and magnesium present in its hexagonal, less dense crystalline structure. Apatite is found in vertebrate bone, dentine, cementum, and enamel. Regardless of the crystal structure and the phylogenetic group in which it occurs, the process of calcification can be considered to be basically the same (Travis, 1960, 1963), although the function may be specifically adapted for different requirements.

In crustaceans, molting is necessary in the apparent growth process. Thus, considerable quantities of calcium and organic constituents have to be resorbed from the exoskeleton prior to ecdysis, but total resorption is limited to certain areas, i.e., the endophragmal skeleton and the ecdysial sutures. After resorption (via the blood) of these constituents, the organism is confronted with an abnormally high concentration of these substances in its internal fluids and the animal must either store or excrete this

excess. If the availability of the resorbed constituent is sparse, the majority of this element is usually stored. Crayfish generally store some of the resorbed calcium and phosphorus as small buttons, called gastroliths, in the lining of the stomach. Gastrolith formation and dissolution have been followed throughout the ecdysis cycle by Damboviceanu (1932), Numanoi (1937), Keyer (1942), Scudamore (1942, 1947), Travis (1955b, 1960, 1963), and McWhinnie (1962). Marine crustaceans generally store some of the resorbed calcium and phosphorus in the mid-gut gland. Paul and Sharpe (1916) have reported that this process occurs in *Cancer pagurus*. This also occurs in *Carcinus maenas* (von Schönborn, 1912; Robertson, 1937), in *Maia squinado* (Drach, 1939), in *Hemigrapsus nudis* (Kincaid and Scheer, 1952) and in the lobster, *Panulirus argus* (Travis, 1955a). Miyawaki and Sasaki (1961) found the same in the fresh water crayfish, *Procambarus*. Calcium is present in relatively high concentrations in sea water and therefore this element may not be a limiting factor in molting, and so not much of it may need to be stored during proecdysis of a marine crab. The concentration of phosphate in Hawaiian waters, however, is small (Sather, 1966), and therefore it would seem to be necessary for the animal to conserve this element to a greater extent than calcium. After ecdysis is completed, the organism would use the resorbed and stored materials for calcification of the new exoskeleton. The amount of inorganic material stored, however, is not sufficient to account for the total amount found in the intermolt crustacean. Therefore, the animal must actively concentrate the elements from the environment.

The molt cycle of crustaceans has been the subject of a great number of investigations. Apart from descriptions of morphological changes, the mineral metabolism has been studied to a certain extent, particularly changes in calcium and phosphorus content (Travis, 1954, 1955b, 1963). But such changes have been investigated only at random periods in the molt cycle, and only in certain tissues and organs (glands). Some emphasis has been placed on the effect of hormonal influences (eyestalk hormones, etc.) on the alterations (Carlisle, 1954; McWhinney, 1962). No data have been available on calcium and phosphorus metabolism throughout the entire molt cycle of a crab, nor has anything been known of the concentrations and distribution of these elements in the animal at times of calcification and decalcification, periods of major importance in the cycle. Therefore, these studies were undertaken on the physiological processes which occur in the molt cycle of the crab, *Podophthalmus vigil*.

MATERIALS AND METHODS

In the period from March 1961 to October 1963, approximately 1,450 specimens of *P. vigil* were collected from Kaneohe Bay, Oahu, Hawaii and transported to the University of Hawaii Marine Laboratory. The animals were sexed, staged, tagged, and placed in aquaria with a continuous supply of fresh sea water. Modifications of the classification schemes of Drach (1939) and Hiatt (1948) were employed to determine the molt stages of *P. vigil*. A descriptive analysis of the molt scheme was presented by Sather (1966). Crabs in the same stage were placed in a specific aquarium. The animals were usually fed pieces of frozen fish twice a week, but occasionally fresh crab muscle or frozen beef liver was substituted.

When a crab reached a desired stage, it was removed from the aquarium and carefully dried with tissue paper. A 1 ml blood sample was taken from the heart by making a small hole with a dental drill in the carapace immediately posterior of the cardiac and mesobranchial suture and inserting a No. 21-gauge hypodermic needle fitted to a syringe into the exposed pericardium. The crab was then killed and rinsed with distilled water. The gills, mid-gut gland, muscle, and carapace were dissected free, and these, together with the "remainder," were placed into separate tared crucibles. After weighing, the crucibles were placed in a drying oven for 12 hours at 114°C. After weighing, they were dry-ashed at 550°C for 24 hours. The fresh, dry, and ashed weights were recorded and the water, organic, and inorganic contents were calculated. Aliquots of the ashed tissues were taken for the determinations of calcium and phosphorus. The blood samples were stored for later chemical analysis. The exuviae were treated in the same manner except that the fresh

weights were not determined because it was not possible to dry thoroughly the gills and endophragmal skeleton.

The flame spectrophotometric analysis of Geyer and Bowie (1961) was used to determine the calcium content of the ashed samples. The blood calcium was determined using the method of Ferro and Ham (1957a, 1957b). The method of Bernhardt, Chess, and Roy (1961) was used to determine the phosphorus (P_2O_5) content in both the ashed and blood samples. All flame spectrophotometric determinations were carried out on a Beckman DU spectrophotometer equipped with a hydrogen-oxygen burner and a photomultiplier. Blank samples were carried throughout the analysis.

The hydration, organic, inorganic, calcium, and phosphorus data were transformed to arcsin values and the latter were statistically analyzed to determine whether interactions between the various parameters were present. The parameters were also subjected to the D-test of Hartley to ascertain the differences among the means (Snedecor, 1959).

RESULTS

Table 1 contains the results of the statistical interaction analysis. The law of probability values indicate that interactions of hydration, organic, inorganic, calcium, and phosphorus contents had occurred, which illustrates that the chemical parameters of the organs did not uniformly fluctuate from one molt stage to another. The interactions demonstrate that the components were being accumulated by the various organs during different stages, which suggests that the constituents were being transferred between organs at different times.

The results of the statistical comparisons of per cent hydration, inorganic and organic, and calcium and phosphorus among the means are incorporated in Tables 2, 3, and 4, respectively. The concentrations of the components in the various organs, throughout the molt cycle, are listed in decreasing order. In Table 2 the appearance of a superscript number in a molt period signifies that the content of the organ at that period is greater than those with a lesser superscript and without a superscript. The contents during molt periods having equal superscripts are not statistically different from each other. For example, the hydration of the mid-gut gland (Table 2) during the C_{3-4} period is significantly greater than that during C_{1-2}, B_{1-2}, A_{1-2}, D_{1-2}, and D_{3-4}. The amounts of mid-gut gland water during the C_{1-2}, B_{1-2}, and A_{1-2} periods are greater than those during D_{1-2} and D_{3-4}; but, the amounts at the C_{1-2}, B_{1-2}, and A_{1-2} periods do not significantly differ from each other.

The ordinates of Figures 1–7 are expressed as per cent content, which is not the most ideal

TABLE 1

INTERACTION ANALYSIS OF PERCENTAGE, COMPOSITION OF FIVE COMPONENTS IN SAMPLED ORGANS OF *P. vigil*

% COMPOSITION	no	F-VALUE	PROBABILITY
Hydration+	16.36	55.33	< 0.01
Organic	15.78	7.56	< 0.01
Inorganic	15.98	27.86	< 0.01
Calcium	15.04	2.86	< 0.01
Phosphorus	11.30	5.86	< 0.01

no = average number in each class.
+ = calculated real values.
f_1, f_2 (degrees of freedom) = 20 and 400, respectively.

TABLE 2

COMPARISON AMONG THE MEANS: PER CENT HYDRATION OF FOUR ORGANS OF *P. vigil* THROUGHOUT THE MOLT CYCLE

ORGAN AND CONCENTRATION IN DECREASING ORDER			
CARAPACE	GILL	MID-GUT GLAND	MUSCLE
A_{1-2}^{5}*	C_{1-2}^{3}	C_{3-4}^{5}	C_{3-4}^{4}
B_{1-2}^{4}	C_{3-4}^{3}	C_{1-2}^{2}	C_{1-2}^{4}
C_{3-4}	B_{1-2}^{3}	B_{1-2}^{2}	B_{1-2}^{2}
C_{1-2}	A_{1-2}^{1}	A_{1-2}^{2}	A_{1-2}^{2}
D_{3-4}	D_{1-2}	D_{1-2}	D_{3-4}
D_{1-2}	D_{3-4}	D_{3-4}	D_{1-2}

* Explanation of superscript numbers:
5 = Significantly greater content than those in the last 5 stages.
4 = Significantly greater content than those in the last 4 stages.
3 = Significantly greater content than those in the last 3 stages.
2 = Significantly greater content than those in the last 2 stages.
1 = Significantly greater content than those in nonsuperscripted stages.

TABLE 3

Comparison Among the Means: Organic and Inorganic Contents of Four Organs of *P. vigil* Throughout the Molt Cycle

ORGAN AND CONCENTRATION IN DECREASING ORDER

CARAPACE	GILL	MID-GUT GLAND	MUSCLE
% ORGANIC			
D_{3-4}	$D_{3-4}{}^{3*}$	$D_{1-2}{}^4$	$D_{1-2}{}^4$
C_{1-2}	$A_{1-2}{}^3$	$D_{3-4}{}^4$	$D_{3-4}{}^4$
C_{3-4}	$D_{1-2}{}^3$	$A_{1-2}{}^1$	$A_{1-2}{}^2$
B_{1-2}	B_{1-2}	$C_{1-2}{}^1$	$B_{1-2}{}^2$
A_{1-2}	C_{1-2}	$B_{1-2}{}^1$	C_{1-2}
D_{1-2}	C_{3-4}	C_{3-4}	C_{3-4}
% INORGANIC			
$D_{1-2}{}^5$	$D_{1-2}{}^4$	$D_{3-4}{}^5$	C_{3-4}
$D_{3-4}{}^2$	D_{3-4}	$D_{1-2}{}^2$	D_{1-2}
$C_{3-4}{}^2$	C_{3-4}	$A_{1-2}{}^1$	C_{1-2}
$C_{1-2}{}^2$	C_{1-2}	$B_{1-2}{}^1$	D_{3-4}
$B_{1-2}{}^1$	B_{1-2}	C_{3-4}	B_{1-2}
A_{1-2}	A_{1-2}	C_{1-2}	A_{1-2}

* For explanation of superscript numbers see legend for Table 2.

TABLE 4

Comparison Among the Means: Calcium and Phosphorus Contents of Five Organs Throughout the Molt Cycle

ORGAN AND CONCENTRATION IN DECREASING ORDER

CARAPACE	GILL	MID-GUT GLAND	MUSCLE	BLOOD
% CALCIUM				
$C_{3-4}{}^{2*}$	$C_{3-4}{}^1$	$D_{3-4}{}^1$	$D_{3-4}{}^1$	$D_{1-2}{}^5$
$D_{3-4}{}^2$	$D_{3-4}{}^1$	$D_{1-2}{}^1$	C_{3-4}	B_{1-2}
$D_{1-2}{}^1$	$C_{1-2}{}^1$	C_{3-4}	D_{1-2}	C_{1-2}
$C_{1-2}{}^1$	$A_{1-2}{}^1$	A_{1-2}	C_{1-2}	C_{3-4}
B_{1-2}	$D_{1-2}{}^1$	B_{1-2}	A_{1-2}	A_{1-2}
A_{1-2}	B_{1-2}	C_{1-2}	B_{1-2}	D_{3-4}
% PHOSPHORUS				
$A_{1-2}{}^5$	D_{3-4}	$B_{1-2}{}^1$	$D_{1-2}{}^1$	$D_{1-2}{}^4$
$B_{1-2}{}^2$	C_{3-4}	$C_{1-2}{}^1$	$A_{1-2}{}^1$	$D_{3-4}{}^1$
$C_{1-2}{}^1$	C_{1-2}	$A_{1-2}{}^1$	C_{1-2}	C_{1-2}
D_{3-4}	B_{1-2}	$C_{3-4}{}^1$	B_{1-2}	A_{1-2}
C_{3-4}	A_{1-2}	$D_{1-2}{}^1$	D_{3-4}	B_{1-2}
D_{1-2}	D_{1-2}	D_{3-4}	C_{3-4}	C_{3-4}

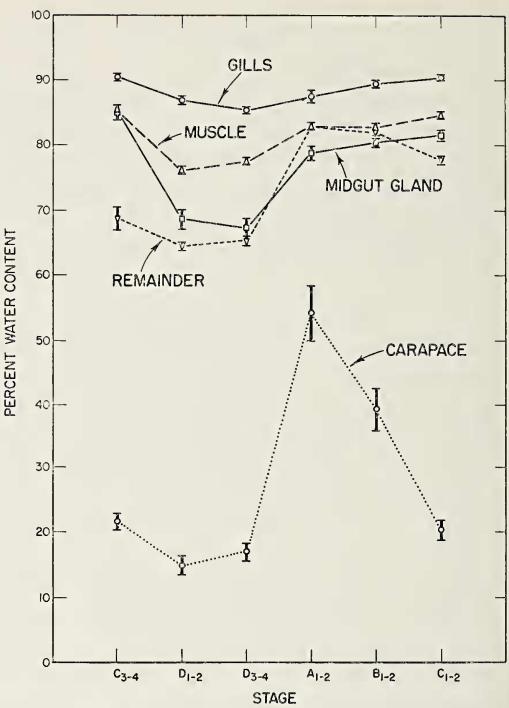* For explanation of superscript numbers see legend for Table 2.

FIG. 1. Changes (mean ± S.E.) in the water content of five organs of *P. vigil* during the molt cycle.

index. Alterations in one component, e.g., organic content, may affect the per cent composition of another component, i.e., inorganic content. Therefore, losses or gains in one particular constituent may only reflect losses or gains in another. Alterations in per cent composition were chosen because the data appearing in most of the literature were expressed in these terms and, thus, this index made comparisons more accessible.

More expressive indices would be: mg or mEq/mg N, or mg or mEq/gm water. The latter ratio is more valid when comparisons of equilibria are desired (Robertson, 1960).

Figure 1 illustrates the alterations in water content of the carapace, mid-gut gland, muscle, gills, and "remainder" during the molt cycle. It is clear that the organs become somewhat dehydrated during proecdysis and rapidly rehydrated during ecdysis.

The organic and inorganic contents of the four organs are plotted in Figures 2 and 3, respectively. The comparable data for the "remainder" were not determined because this por-
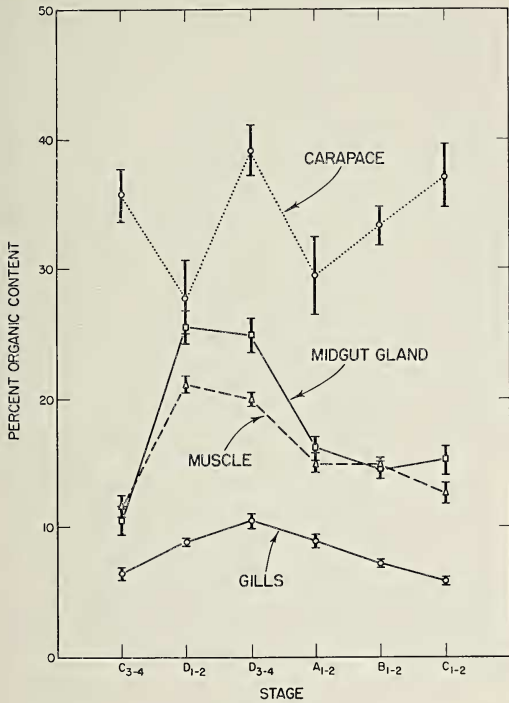
FIG. 2. Changes (mean ± S.E.) in the organic content of four tissues of *P. vigil* during the molt cycle.

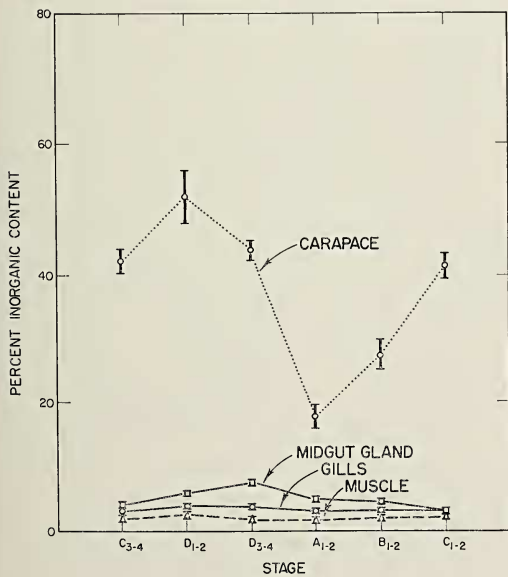tion was composed primarily of exoskeleton, and so the values would probably approximate those of the carapace. It is apparent that the organic contents of the mid-gut gland, gill, and muscle increased during the proecdysial stages. The greatest inorganic fluctuation was found in the carapace. Only minor alterations were found in the other tissues.

The calcium and phosphorus composition of the carapace, mid-gut gland, gills, muscle, and blood were determined. The results, based on dry weight, are plotted in Figures 4–8. In Figures 4–7, the data are plotted as changes in per cent dry weight. The values for the blood (Fig. 8) are presented as mM/liter.

The organic and inorganic composition of the exuviae were also determined. The results are illustrated in Figure 9. Also contained in this figure are the calcium and phosphorus contents of the exuviae, expressed as per cent composition.

The organic, inorganic, and calcium contents of the entire exuvia were compared with those found in the exuvial carapace; this information is summarized in Table 1. The carapace contained less organic material and less calcium than the entire exuvia. The mount of inorganic
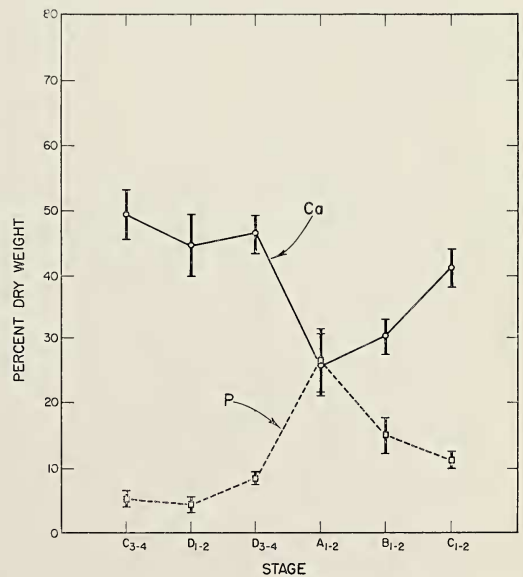

FIG. 3. Changes (mean ± S.E.) in the inorganic content of four tissues of *P. vigil* during the molt cycle.

FIG. 4. Calcium and phosphorus content (mean ± S.E.) of the carapace of *P. vigil* during the molt cycle.

FIG. 5. Calcium and phosphorus content (mean ± S.E.) of the gills of *P. vigil* during the molt cycle.

FIG. 7. Calcium and phosphorus content (mean ± S.E.) of the mid-gut gland of *P. vigil* during the molt cycle.
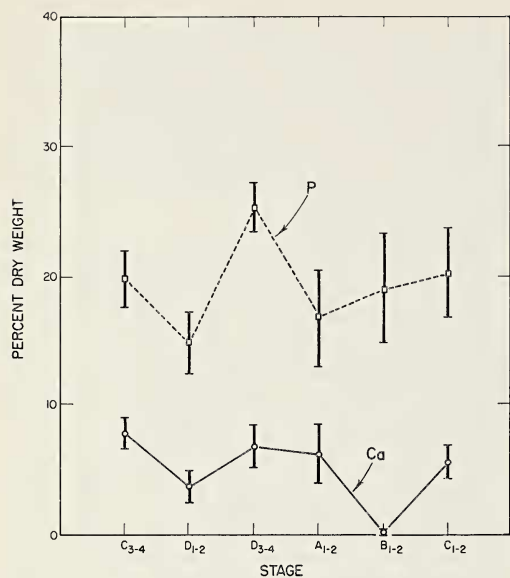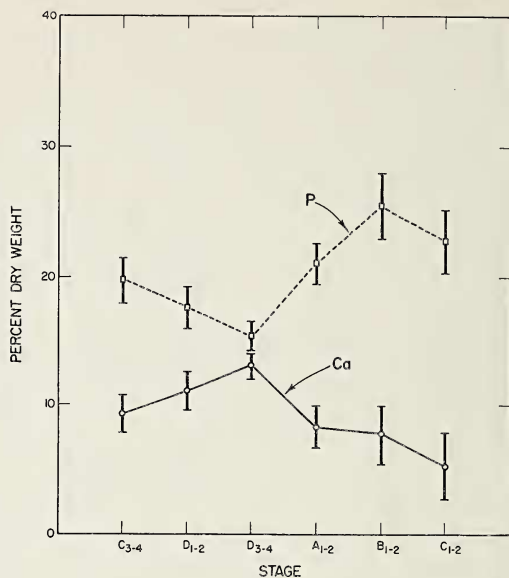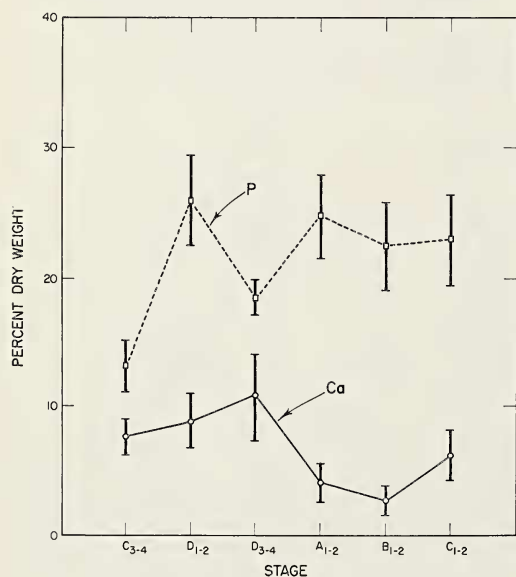

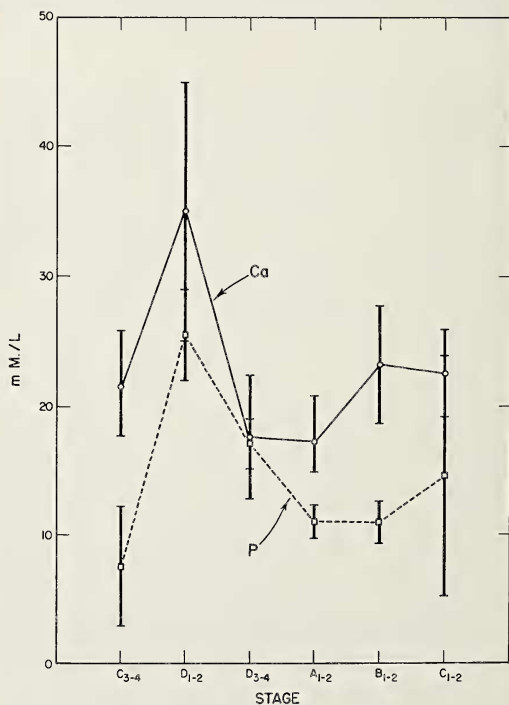FIG. 6. Calcium and phosphorus content (mean ± S.E.) of the muscles of *P. vigil* during the molt cycle.

FIG. 8. Calcium and phosphorus content (mean ± S.E.) of the blood of *P. vigil* during the molt cycle.
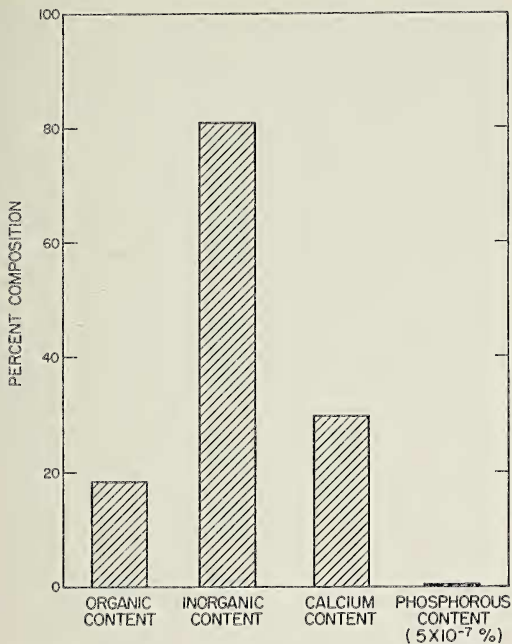
FIG. 9. Percent composition of the exuvia of *P. vigil* (values based on dry weight).

matter in the carapace was greater than that in the entire exuvia.

To determine whether larger crabs were able to resorb the same amount of calcium as smaller crabs, the amount of calcium in the exuviae was plotted against exuvial carapace width. (The data were then statistically analyzed for regression and the slope was fitted by the least squares method.) Figure 10 clearly indicates that as the crabs increased in size the amount of resorbed calcium decreased, and the regression analysis showed that the calculated slope was 0.082 (P <0.001).

DISCUSSION AND CONCLUSIONS

Weight Changes During the Molt Cycle

Changes in weight of Crustacea during ecdysis are due to absorption of water (Baumberger and Olmsted, 1928; Drach, 1939; Needham, 1946; Guyselman, 1953; Travis, 1954). The alterations of body weight and water content of *P. vigil* have been reported elsewhere (Sather,

FIG. 10. Regression of calcium content of exuviae on exuvial carapace widths of *P. vigil*. b = 0.082 (P < 0.001). Upper and lower curves represent the 95% confidence limits.

1966). In brief, during proecdysis the crabs have a tendency to lose weight, which can be attributed to a loss of water. Between D_3 and 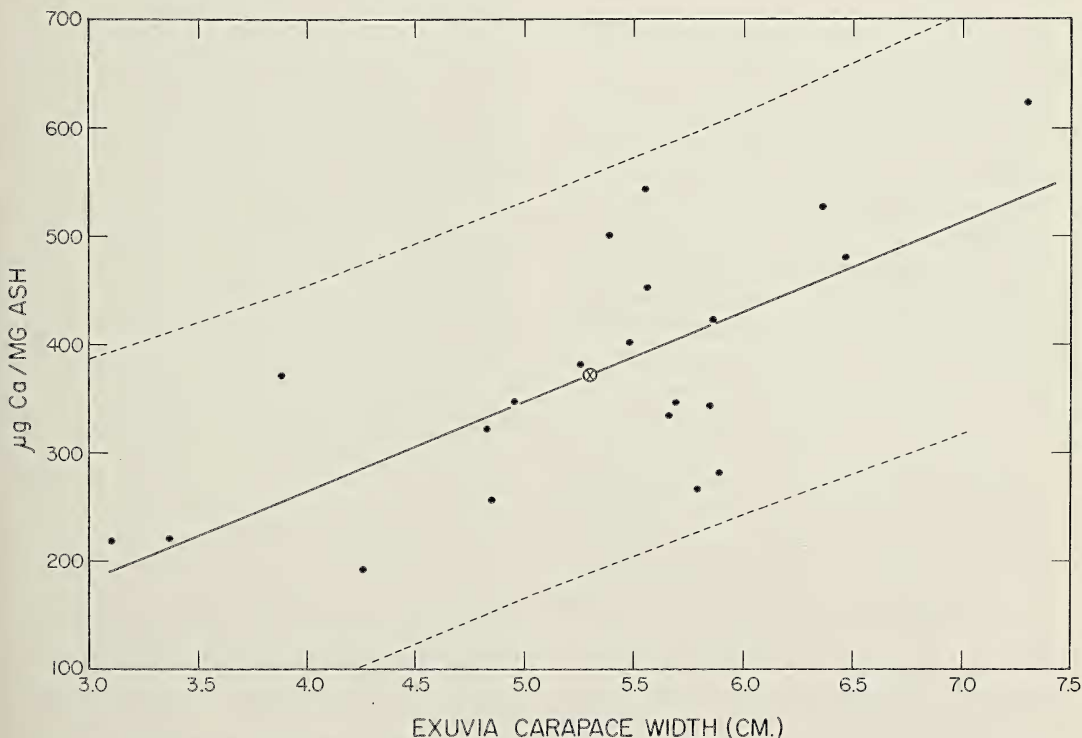D_4 the mean weight gain was about 8%. The weight change between D_4 and A_1 was an insignificant loss of 1.5%. However, the crabs gained 18.8% between A_1 and A_2. No significant weight alterations were noted between A_2 and B_1, B_1 and B_2, and B_2 and C_1. Between stages C_1 and C_2 the weight gain was 4.4%. No significant changes were found during the remainder of the molt cycle. However, the overall weight gain between two successive intermolt stages was approximately 34%.

The total water content of the crabs during the ecdysis cycle was also followed. During the premolt stages, the crabs tend to become dehydrated; the water lost at D_{1-2} and D_{3-4} was calculated to be 8.1% and 7.7% below the C_4 water content of 70%. The postmolt water content was increased from about 62% (D_{3-4}) to 77% (A_{1-2}) and 75% (B_{1-2}), but these water content changes were statistically not significantly different from the intermolt value of 70.3%.

Water Content of Four Organs and "Remainder" Throughout the Molt Cycle

The amount of water in the various tissues of a crustacean during the molt cycle has not been previously reported. The water content of the carapace, mid-gut gland, gills, muscles, and "remainder" in *P. vigil* is illustrated in Figure 1. (The values are the means ± S.E.) Analysis of variance on the arcsin transformed values illustrated that interaction was present, showing that the water content of the tissues did not vary uniformly throughout the molt stages.

The greatest fluctuations in water content were found in the carapace during ecdysis. A decrease of about 5% was noted during the proecdysial stages, but this was not statistically significant. The gain in water content between D_{3-4} and A_{1-2} was a significant gain of about 37%. Although the extracellular fluid volumes were not determined, this gain could possibly reflect a greater extracellular fluid volume. During the B_{1-2} and C_{1-2} stages, the water content was decreased to 20.54%, which was caused by the incorporation of calcium salts.

The alterations in gill hydration are represented as the top curve in Figure 1. During the premolt stages, the gills lost 4.57% of their water content—a significant decrease. After ecdysis the gill water content was increased to about 87.64%, which was found to be a significant gain. The hydration at B_{1-2} was increased to a significant 89.42%. The per cent hydration of the gills during the C_{1-2} period was not significantly different from that at the C_{3-4} duration (90.20%).

Robertson (1960) has demonstrated that the gills of *Carcinus maenas* were the site of water and ion absorption but that the antennal glands were the sites for the loss of the water and the ions. Because the urine of *P. vigil* was not sampled, it is not possible to exclude these glands and the gills as the sites of water flux.

The decrease in water content of the muscles during the premolt stages from a value of 85% to 76% was found to be significant, as was the increase to 82% at the A_{1-2} periods. The further increases during postecdysis were not significant.

The same type of pattern is seen to occur in the mid-gut gland. The intermolt water content was found to be 85.16%. The reduced hydration during the premolt stages to about 68% was a significant drop. During the A_{1-2} duration, the water content was increased to 78.24%. The subsequent changes observed during postmolt did not differ significantly.

Excluding the carapace and the remainder, the increase of about 11% in the mid-gut gland during ecdysis was the greatest alteration. Robertson (1960) reported that in *C. maenas* the water content of the mid-gut gland and its fluid increased during the early postmolt stages. This was attributed to absorption of water via the fore-gut. The results reported here for *P. vigil* are consistent with those reported by Robertson (1960) and also with the findings of Drach (1939) for *Maia squinado* and *Cancer pagurus*.

The fluctuations of the "remainder" during the molt cycle are also illustrated in Figure 1. It is quite obvious that this portion also lost some water during proecdysis. The intermolt water content was calculated to be 68.69%, and postecdysial values of about 65% were significantly different from the former. After ecdysis the water content rose to a significant high of 83.18%. No statistical difference was found

between the A_{1-2} and B_{1-2} values. During the last two postecdysial stages, the water content decreased to 77.78%. Because the "remainder" was largely composed of exoskeleton, this curve follows the same general pattern as the carapace. The increase in hydration following ecdysis was probably due to a greater extracellular fluid volume of the exoskeletal tissues. During calcification, the extracellular water was undoubtedly replaced by calcium salts.

The water content of the whole blood during the premolt stages of *P. vigil* was not determined. Water is not absorbed during the first three proecdysial stages (Sather, 1966). Travis (1954, 1955*b*) demonstrated that the premolt water uptake by *Panulirus argus* was limited to 15 minutes just prior to ecdysis. However, as can be seen in Figure 1, *P. vigil* becomes desiccated during proecdysis. The water content of the intermolt blood was found to be 94.14%. The value for the A_{1-2} stages was 93.98%; at B_{1-2}, 91.46%; and at the C_{1-2} period, 93.88%. Because the greatest absorption of water occurs during the period immediately following ecdysis, it would be expected that the blood at the A_{1-2} duration would be somewhat diluted (to be explained below). Travis (1955*b*) found that the blood calcium and phosphorus concentrations of *P. argus* were decreased following molt. Robertson (1960) reported that in *C. maenas* the concentrations of blood constituents were reduced during the early postmolt stages. Both authors attributed their findings to the uptake and retention of water.

Proecdysial and early postecdysial *P. vigil* exhibit significant alterations in water content. This phenomenon offers a fine thesis problem on the osmoregulatory mechanisms and modes employed by the crab during this "stressed" duration.

Organic Content of Four Organs Throughout the Molt Cycle

The organic content of the carapace, gills, muscle, and mid-gut gland are plotted in Figure 2. Analysis of variance illustrated that the organic content of the organs did not uniformly fluctuate.

A great variability is seen in the organic content of the carapace, but none of the alterations

was found to be significant from the intermolt content of 35.76%.

The organic composition of the mid-gut gland increased from an intermolt value of about 10% to approximately 25% at the premolt stages. This accounts for the hyperglycemia reported for proecdysial *Panulirus japonicus* by Scheer and Scheer (1951). After ecdysis, the organic value dropped to 16.15%, which can be similarly attributed to the loss of reserves utilized for the active process of molting and for the tanning of the pigmented layer, especially as the crabs do not feed until the late B stage. The value found at the C_{1-2} period was not significantly different from that of the previous period, but it was greater than that of the C_{3-4} stages. During the early C stages, *P. vigil* becomes voracious, obviously to compensate for the loss which occurred during ecdysis.

The alterations in the organic content of the muscle were similar to those of the mid-gut gland. From an intermolt level of 11.66%, the organic content increased to about 20% during proecdysis. The significant increase in organic material during these periods probably is due largely to the accumulation of glycogen to serve as an energy source for the epidermal cells which form the proecdysial tissues and the molting fluid. It is quite possible that the muscle organic constituents would be at a high level just prior to ecdysis, as the active process of exuviation would require a great amount of energy. In addition, crabs in the early D_3 stage did feed and the resulting energy would have to be stored either in the mid-gut gland or in some other tissue. Undoubtedly, the former has a saturation level and the most likely tissue, therefore, would be the muscle because of its adequate blood supply.

The lowest curve in Figure 2 illustrates the changes in the organic content of the gills. The minimum concentration of organic materials occurred in the intermolt period (6.51%) and the highest (10.45%) was found to occur in the D_{3-4} stages. The premolt gains were significantly greater than the intermolt value. During the postecdysial stages, the gill organic content decreased to the original intermolt content.

One possible explanation for the observed increase in gill organic content at D_{3-4} would be

that a supply of energy would be required to regulate the ionic ratio of the internal to external media. Unfortunately, the glycogen content of the gills was not determined. Considering the decreased water content of *P. vigil* during late premolt, it appears that the gills may be possible sites of water and/or ion efflux. This hypothesis is not in agreement with the report by Robertson (1960), which stated that the antennal glands of *C. maenas* were the sites of water and ionic efflux. One possible method for determining the site of water flux in *P. vigil* would be the use of tritiated water. Radioisotopes representing the extracellular ions would also verify the sites of ionic flux.

Because the organic content of premolt blood was not determined, it was not possible to follow the changes throughout the ecdysis cycle. The organic constituent during the A_{1-2} period was 2.93% of the whole blood. The content in stages B_{1-2} was 4.02%; in the C_{1-2} stages, 2.86%; and in the C_{3-4} stages, 2.64%. The observed increase during the B period may be due to the commencement of food ingestion and the subsequent loss (during the C stages) by the distribution to the mid-gut gland and exoskeleton.

Inorganic Content of Four Organs Throughout the Molt Cycle

The fluctuations in inorganic content are illustrated in Figure 3. Analysis of variance indicated that interaction was again present.

As could be expected, the carapace varied much more in inorganic content than did the other sampled tissues. The intermolt value was determined to be 42.12%. The significant increase to 52.01% during the D_{1-2} stages possibly can be attributed to the formation and tanning of the new epicuticle. However, the inorganic constituent in the proecdysial tissues is not dominantly calcium (see Fig. 4). The finding is in agreement with those of other investigators (Drach, 1936, 1939; Krishnan, 1950; Travis, 1955a, 1960, 1963). During the D_{3-4} stages when resorption was nearly completed, the inorganic content was decreased significantly to 43.80%. It becomes apparent that the entire carapace is not an area where maximum resorption occurs (see introductory remarks). Following ecdysis the inorganic content

of the carapace was approximately 20%, reflecting the amount found in the exocuticle. During the A_{1-2} periods, the exocuticle was impregnated with calcium salts, with concomitant formation of the principal layer. During the B_{1-2} and C_{1-2} stages, periods of major calcification, the inorganic composition was increased to 27.31% and 41.28%; both were significant gains. The amount during the early C periods approximated that of the intermolt content.

The mid-gut gland's alterations are plotted also in Figure 3. The increased values during premolt ($D_{1-2} = 5.98\%$, $D_{3-4} = 7.74\%$) were statistically different from each other and also from the intermolt value of 4.29%. This was probably due to the storage of some constituents absorbed from the exoskeleton. Similar storage has been reported by Paul and Sharpe (1916) for *Cancer pagurus;* by von Schönborn (1912) and Robertson (1937, 1960) for *Carcinus maenas;* by Drach (1939) for *Maia squinado;* by Kincaid and Scheer (1952) for *Hemigrapsus nudus;* and by Travis (1955a) for *Panulirus argus.* The reduction in inorganic content during the postmolt stages can be attributed to the redistribution of the stored elements to the hardening exoskeleton.

The gill inorganic content was found to vary slightly. The observed value of 4.15% at the D_{1-2} duration was found to differ significantly from those of the other durations. The greater inorganic content was due to the formation of the proecdysial tissues, which similarly occurred in the carapace, i.e., the epicuticle and exocuticle.

As expected, the mean per cent inorganic composition of muscle did not vary significantly between stages ($P > 0.05$). The average content of the inorganic materials was 2.44%, the range being 2.14–2.69%.

The intermolt inorganic composition of the whole blood was 3.22%. During the A_{1-2} period it was 3.09%, and at the B_{1-2} it was 4.52%. The former value reflected the absorption of sea water, and the latter value was probably due to the mobilization of calcium, after it was actively transported by the gills to the exoskeleton. The blood inorganic content at the C_{1-2} period was approximately equal to the intermolt value, indicating that the sclerotinization process was nearly completed.

Calcium and Phosphorus Contents of Five Organs Throughout the Molt Cycle

The calcium and phosphorus contents of the carapace, gills, mid-gut gland, muscle, and blood were determined. The values, based on per cent dry weight, are plotted in Figures 4–8. The blood data are given in mM/liter. Interaction analyses on the organs' calcium and phosphorus contents were positive (P < 0.01).

The intermolt carapace calcium content (Fig. 4) was 49.51% and the phosphorus content was only 5.42%. Both of these values are large in comparison with those reported by Prenant (1928) for five species of crabs in temperate waters. His data (per cent calcium and phosphorus, respectively) were: *Carcinus maenas*, 30 and 2; *Maia squinado*, 31 and 2; *Portunus puber*, 36 and 2; *Cancer pagurus*, 36 and 0.8; and *Xantho floridus*, 38 and 0.4. However, as noted by Vinogradov (1953), the majority of these values were only relative. Hayes, Singer, and Armstrong (1962) reported that the carapace calcium of the lobster, *Homarus vulgaris*, was 25.2% and the calcium content of the claw was 23.8%. The phosphate contents of these two anatomical areas were reported to be 1.33% and 2.05%. The lower calcium content of temperate species may be a genetic difference or may be caused by a lesser availability of this environmental element. Unfortunately, this latter possibility cannot be checked because calcium data at the collection sites were not available. The environmental calcium content certainly influences the amount absorbed by an organism, as well as the amount retained. It is known that the total calcium content of fresh water crustaceans is less than that in marine species.

The calcium alterations of the carapace during the molt cycle are illustrated in Figure 4. Preceding molt, the calcium content varied only slightly during the D periods. After ecdysis (A_{1-2}) the calcium content was diminished to 26.04% and reflected the amounts in the epicuticle and pigmented layers. Between B_{1-2} and C_{1-2}, which was the major duration of calcification, the calcium content was increased significantly to 41.12%, which was similar to that of intermolt.

The phosphorus changes of the carapace are also illustrated in Figure 4. The content at C_{3-4} was calculated to be 5.42%, which is much greater than that found in *Panulirus argus* by Travis (1957). In *P. argus,* in late stage C, about 3% of the total integument was composed of $Ca_3(PO_4)_2$, which is approximately 0.2% of the total phosphorus. In *P. vigil,* a small insignificant increase was observed during the last proecdysis stages. At stages A_{1-2}, the phosphorus content was increased to 26.76% of the dry weight, which was about the same as the calcium concentration. This localization could have been due to the mobilization of phosphorus by the blood. Travis (1957) has demonstrated that the postecdysial integument stains heavily for alkaline phosphatase. Thus, a much greater amount of phosphorus would be present than during the other stages. It is thought that alkaline phosphatase liberates phosphates which combine with calcium to form the calcium phosphate complex. The high phosphate content during the A stages causes one to ponder over its significance, because the major anionic constituent of the intermolt integument is carbonate and not phosphate. In *P. vigil* during the first C periods, the phosphorus content decreased significantly to 11.34%. This reduction can be attributed to the increased deposition of calcium salts.

The fluctuations in gill calcium are plotted in Figure 5. No significant differences were found between the intermolt value of 7.88% and the first premolt values. However, the decreased value of 0.30% at B_{1-2} did differ significantly from the other values. Because the B_{1-2} period is the initial duration of greatest calcification, a high gill permeability, caused by calcium, would be greatly detrimental for extraction of calcium from the medium. Robertson (1960) demonstrated that a great influx of calcium occurred during postmolt in *Carcinus maenas*. Unfortunately, the amount of calcium in the gills was not measured. It appears, then, that in *P. vigil* the mechanism to increase the movement of calcium into the animal serves to reduce the gill calcium content, allowing calcium to enter across the gills at a more rapid diffusion rate, the blood then mobilizing the element to the integument.

The phosphorus content of the gills during the molt cycle is illustrated in Figure 5. The

intermolt value was calculated to be 19.80% of the dry weight. Analysis of variance illustrated that there were no significant differences among the means. However, there is a suggestion that the gills may store phosphorus during the D_{1-2} stages. This suggestion is reinforced by the gill organic content at D_{1-2} (Figure 2). The phosphorus content may be indicative of an energy-requiring process for early postecdysial absorption of sea water.

Figure 6 demonstrates the alterations of calcium and phosphorus content of the muscle throughout the molt cycle. The calcium concentration increased from 7.70% at the C_{3-4} period to 10.72% at the D_{3-4} stage. Following ecdysis the calcium content was reduced to 4.14%, and at the B_{1-2} period it was further reduced to a significant 2.72%. During the period when the majority of calcification occurred (between B_{1-2} and C_{1-2}), the muscle calcium was raised to 6.28%.

During the process of exuviation, i.e., the time when active musclar contractions occur to facilitate withdrawal of the crab from the old exoskeleton, a large amount of energy would be required. As can be seen in Figure 2, the organic content of this organ also increased. Another requirement would be an ample supply of calcium to expedite muscular contractions. Calcium and organic reserves may be localized in this organ to insure proper exuviation. This phenomenon would then favor the survival of the crab during the process of ecdysis. The muscles, in addition to the mid-gut gland, may also serve as a place for calcium storage.

The 6.58% decrease in calcium between the D_{3-4} and A_{1-2} periods may be due to the mobilization of the element to the exocuticle; during the first postecdysial stages, the latter is impregnated with and concomitantly hardened by calcium salts (Travis, 1960, 1963).

The muscle phosphorus content was greater than that of calcium; phosphorus is the most important element in muscle contraction. The values at D_{1-2} (25.98%) and A_{1-2} (24.82%) were significantly different from the intermolt content of 13.14%, but the former values were not statistically different from each other. The loss of phosphorus during D_{3-4} may have been due, in part, to the mobilization to the gills and carapace, but 2.67% cannot be accounted for.

The reasons discussed in the above paragraph seem to be applicable to phosphorus as well as to calcium. However, the suggestion that proecdysial storage of phosphorus in the muscles occurred is indeed very weak.

On examination of Figure 6, it can be seen that phosphorus and calcium are controlled differently. After ecdysis the amount of calcium in the muscles is diminished, but the phosphorus content remains relatively constant.

The phosphorus and calcium fluctuations in the digestive gland are seen in Figure 7. As found in the alterations in carapace calcium and phosphorus (Fig. 4), the curves tend to be reciprocal to each other. But the mid-gut gland phosphorus content was always greater than that of calcium. Significant differences among the means did exist ($P < 0.01$).

The intermolt calcium content of 9.31% did not differ significantly from that at D_{3-4} (13.05%). However, the gain suggests that some calcium is stored in the mid-gut gland during premolt. After ecdysis some of the calcium (5% in $P.$ $vigil$) may be used for calcification of the exocuticle. These observations are consistent with the reports of Paul and Sharpe (1916), von Schönborn (1912), Drach (1939), Kincaid and Scheer (1952), and Travis (1955a), but are inconsistent with that of Robertson (1960), who reported that in $C.$ $maenas$ the mid-gut gland secretion during postmolt (stages A and B) had about 16% more calcium than it did during intermolt.

The phosphorus content decreased from 19.76% in C_{3-4} to 15.28% in the late D stages and increased to 21.04% and 25.50% during the A_{1-2} and B_{1-2} stages, respectively. The postecdysial gain may have been due to mobilization from the gills (Fig. 5), which lost approximately 10% during postmolt. It is obvious that this gland in $P.$ $vigil$ does not store phosphorus during the premolt periods, but it does appear that the gland becomes a phosphorus reservoir after ecdysis. This finding is inconsistent with the reports by Travis (1955b, 1957) that phosphorus was stored in the midgut gland of $P.$ $argus$ prior to ecdysis, but that following molt, the phosphorus content rapidly decreased. The latter conclusion was based primarily on histochemical observations and

blood analysis, and no chemical analysis of the mid-gut gland was undertaken.

A question arises after examining all of the phosphorus curves. Because the crabs were not feeding during the A and early B stages, where did the phosphorus originate? From Figure 5 it is seen that the phosphorus content of the gills is drastically increased during the D_{3-4} stages. Following ecdysis the phosphorus content of the organ was reduced by approximately 9%. The phosphorus content of the mid-gut gland from D_{3-4} to A_{1-2} was increased by about 6%. Therefore, possibly the gills, rather than the mid-gut gland, serve as a phosphorus reservoir. Another possible source for the accumulation of phosphorus could be the water that was imbibed immediately following ecdysis. However, this is not likely because a pilot experiment demonstrated that phosphorus is not accumulated during and following ecdysis. The application of the radioisotoype P^{32} could be very useful in resolving this question.

Figure 8 illustrates the calcium and phosphorus contents of the blood during the molt cycle. The data are plotted on a volume basis, as is shown on the ordinate. The blood calcium and phosphorus levels tend to be parallel throughout the molt cycle.

During the D_{1-2} periods, the blood calcium was significantly increased to 35.09 mM/liter from the C_{3-4} content of 21.58. A significant decrease to 17.68 mM/liter was observed at the D_{3-4} stages. In *Panulirus argus,* Travis (1955*b*) also noted a premolt blood calcium increase, followed by a decrease in the late premolt stages. The loss was attributed to dilution when the lobster took in water. During the A period, the lobster's blood calcium was at the intermolt value. The content was slightly increased at the B stages and, following this interval, i.e., during the C period, the concentration was decreased below the intermolt value. In late premolt *Carcinus maenas,* Robertson (1960) also noted a blood calcium increase of about 21%. Within 24 hours after molting (Stage A), the blood calcium content was reduced by approximately 25%. In 2 to 14 days following ecdysis, the blood calcium was further reduced to approximately 31% of the intermolt value.

The water content of *P. vigil* decreases during proecdysis (Sather, 1966). Also, as evidenced from inspection of the changes in water content of sampled organs (Fig. 1), dehydration definitely occurred during the premolt stages. The first decrease in water content was found during the D_{1-2} period. This would account for the rise in the blood calcium at this interval. The observed reduction at the D_{3-4} stages is not due to the uptake of water. On a volume basis, the amount lost was calculated at 17.41%. However, on a dry weight basis, this loss was only 2.04%. The blood must have lost some calcium to other organs or the external medium. Thus, the calcium may have been distributed to the muscles and/or the mid-gut gland. The large standard errors ($\pm$ 1.2%) for the latter two organs do not permit an accurate estimate of the quantity accumulated by each organ.

Following ecdysis, no significant changes in the blood calcium were observed. As seen in Figure 1, the greatest increase in water content occurred at this time. It should be recalled that the water taken in was sea water, including the elements present in the medium. This has been verified by the studies of Robertson (1960). Thus, a great calcium dilution would not be expected. Also, as seen in Figures 6 and 7, the mid-gut gland and muscle lost some calcium which could have been accumulated by the blood. At period B_{1-2} the blood calcium was increased to 23.23 mM/liter. This could have been due to absorption of calcium, via the gills, from the environment. Figure 5 illustrates that at this interval the gill calcium was drastically reduced, increasing the efficiency of extracting calcium from the external medium.

Thus, except for the effect of dehydration at the early proecdysial stages, the calcium content of *P. vigil* blood remains more stable than in other investigated crustaceans. This fact may be due to the nearly chemically constant environment of the crab. Except for one month, the environmental salinity and calcium was not less than 34 0/00 and 300 mg/liter, respectively (Sather, 1966).

The total phosphorus fluctuations of the blood during the molt cycle are also depicted in Figure 8. The significant increase of blood phosphorus to the D_{1-2} interval of 25.52 mM/ liter can be attributed to the desiccation of the animal. The reduction found at D_{3-4} (17.19

mM/liter) possibly reflects the relocation of phosphorus to the gills (Fig. 5). At stage A_{1-2}, the value was slightly greater than the intermolt value. This decrease can be assigned to dilution, i.e., by the uptake of water from the environment. In sea water, phosphorus is present in much smaller quantities than is calcium. The annual average phosphate content of sea water was less than 1 µg/liter (Sather, 1966). Also, the results of a preliminary experiment illustrated that the phosphorus content of the external medium was increased when containing molting and postmolt crabs. The leveling-off of the blood phosphate at B_{1-2} and the increase at C_{1-2} was probably due to the resumption of feeding. The majority of *P. vigil* began to feed at B_2 and only occasionally when they were in the B_1 stage.

Homarus americanus (Hollett, 1943), *Panulirus argus* (Travis, 1955*b*), and *Carcinus maenas* (Robertson, 1960) also increased their blood phosphorus during the premolt stages. Travis (1955*b*) found that after ecdysis, the phosphorus content steadily decreased. This was attributed to a depletion of phosphorus by calcification of the exoskeleton concomitant with a reduction in the stored mid-gut gland phosphorus. Robertson (1960) reported that within 24 hours after ecdysis the blood phosphorus of *C. maenas* was slightly less than the intermolt value. Within 2 to 14 days after molting the value was increased to about 22% above that of the intermolt level. The report of Drilhon (1935) is not consistent with the above reports, in that the phosphorus content of the blood of premolt and postmolt *Maia squinado* was not altered.

Composition of the Exuviae

The discarded exoskeleton or exuvia of *P. vigil* is not consumed by the crab as it is in the insects. Robertson (1937) reported that the exuvia of *Carcinus* comprised about 46.2% of the total dry weight. Lafon (1948) stated that the value was 47.5% of the dry weight. These calculations were not made on the exuviae of *P. vigil,* but the percentages of organic, inorganic, calcium, and phosphorus contents were determined and these data are given in Figure 9. The per cent composition was based on the dry weight. The histogram illustrates

that about 81% of the entire exuvia was composed of inorganic material and only approximately 37% of the inorganic content was due to calcium. Odum (1957) reported that the calcium content of the exuviated chela of *Uca pugnax* was 27.7% of the dry weight, which is somewhat consistent with the calcium content of the exuvia of *P. vigil.*

Knowing the amount of calcium in the exuvia (30%) and assuming that the intermolt carapace, which contained about 50% calcium, is representative of the entire exoskeleton, it is possible to calculate the quantity of calcium resorbed, which is about 20%. The amount of calcium stored in the mid-gut gland and the muscle was approximately 7%. Thus, the quantity stored and resorbed closely approximates the amount of calcium (26%) present in the early postmolt carapace. The increase in amount (23%) between A_{1-2} and C_{3-4} undoubtedly is acquired from the environment.

The phosphorus content of the exuvia was found to be only approximately $5 \times 10^{-7}\%$ of the dry weight. Unfortunately, comparable phosphorus data for other species have not been reported. Employing the above mathematical deductions, it would seem possible to account for the phosphorus budget. However, such a process produces a deficit of about seven magnitudes. It is possible that the reproductive system and the gastrointestinal tract, which were not sampled, may have been highly selective for the storage of this element. The great resorption of phosphorus is obvious and it must have been stored, because the results of an experiment showed that proecdysial and postecdysial crabs lose very little phosphate (2.6–5.1 µg PO_4) to the environment.

The organic content of the exuvia was found to be only 18.7%. This organic material has been reported to be composed of lipo-protein, chitin, mucopolysaccharides, and proteins (Travis, 1955*a*, 1957, 1963; Dennell, 1960). The amount of organic material resorbed was about 17%, which is consistent with the amounts stored in the mid-gut gland and muscle.

Neto (1943) reported that the calcium content of the carapace of *Uca maracoani* decreased as its breadth increased. The calcium content of the exuviae of *P. vigil* was compared with the width of the cast exoskeletons. Figure 10

clearly demonstrates that less calcium is resorbed from the exoskeleton as the crab increases in size. The calculated slope was found to be 0.082, which indicates an increase of approximately 82 µg Ca/mg ash for each centimeter increase in breadth. The slope differed significantly from 0 (P < 0.001). A similar analysis was performed, comparing the amount of carapace calcium with the wet weight of the C_4 crabs. The results, which are not illustrated in this report, demonstrated that regression did not occur and that the calculated slope was 0.0009. Thus it appears that less calcium is resorbed by large crabs and, therefore, more calcium appears in the exuvia. This phenomenon illustrates the effects of ageing on one physiological process of an invertebrate. It may be possible that the enzymatic activities responsible for crustacean decalcification are decreased with age and those required for recalcification are not influenced by senescence. A study of the alkaline phosphatase and carbonic anhydrase activities of the epidermal cells may verify the above observations.

REFERENCES

BAUMBERGER, J. D., and J. M. D. OLMSTED. 1928. Changes in osmotic pressure and water content of crabs during the molt cycle. Physiol. Zool. 1:545.

BÉ, A. W. H., and D. B. ERICSON. 1963. Aspects of calcification in planktonic Foraminifera (Sarcodina). In: Comparative biology of calcified tissue. Ann. N. Y. Acad. Sci. 109(1):65–81.

BERNHARDT, D. N., W. B. CHESS, and D. ROY.

1961. Flame photometric determination of phosphorus in the presence of sodium, potassium and other cations. Anal. Chem. 33:395.

CARLISLE, D. B. 1954. On the hormonal inhibition of moulting in decapod Crustacea. J. Mar. Biol. Assoc. U. K. 33:61.

DAMBOVICEANU, A. 1932. Composition chimique et physicochimique du liquide cavitaire chez les crustacés décapodes (Physiologie de la calcification). Arch. Roum. Path. Exptl. Microbiol. 5:239.

DENNELL, R. 1960. Integument and exoskeleton. Chap. 14, pp. 449–472. In: T. H. Waterman, ed., Physiology of Crustacea, Vol. 2. Academic Press, New York.

DRACH, P. 1936. L'eau absorbée au cours de l'exuviation, donnée fondementale pour l'étude physiologique de la mue. Définitions et déterminations quantitatives. C. R. Acad. Sci. 202:1817.

——— 1939. Mue et cycle d'intermue chez les crustacés décapodes. Ann. Inst. Oceanogr. 19:103.

——— and M. Lafon. 1942. Étude biochimique sur le squelette tégumentaire des décapodes brachyoures (variation au cours du cycle d'intermue). Arch. Zool. Exptl. 82:100.

DRILHON, A. 1935. Étude biochimique de la mue chez les crustacés brachyoures (*Maia squinado*). Ann. Physiol. Physicochim. Biol. 11:301.

ENNEVER, J. 1963. Microbiologic calcification. In: Comparative biology of calcified tissue. Ann. N. Y. Acad. Sci. 109(1):4–13.

FERRO, P. V., and A. B. HAM. 1957a. A simple spectrophotometric method for the determination of calcium. Am. J. Clin. Pathol. 28:208.

——— ——— 1957b. A simple spectrophotometric method for the determination of calcium. II. A semimicro method with reduced precipitation time. Am. J. Clin. Pathol. 28:689.

GEYER, R. P., and E. J. BOWIE. 1961. The direct determination of tissue calcium by flame photometry. Anal. Biochem. 2:360.

GREENFIELD, L. J. 1963. Metabolism and concentration of calcium and magnesium and precipitation of calcium carbonate by a marine bacterium. In: Comparative biology

of calcified tissue. Ann. N. Y. Acad. Sci. 109(1):23–45.

GUYSELMAN, J. B. 1953. An analysis of the molting process in the fiddler crab, *Uca pugilator*. Biol. Bull. 104:115.

HAYES, D. K., L. SINGER, and W. D. ARMSTRONG. 1962. Calcium homeostatic mechanisms and uptake of radioisotopes in the lobster. Am. J. Physiol. 202(2):383.

HIATT, R. W. 1948. The biology of the lined shore crab, *Pachygrapsus crassipes* Randall. Pacific Sci. 2:135.

HOLLETT, A. 1943. Relation between moult cycle and phosphorus content of blood and muscle. J. Fish. Res. Bd. Canada 6:152.

ISENBERG, H. D., L. S. LAVINE, M. L. MOSS, D. KUPFERSTEIN, and P. E. LEAR. 1963. Calcification in a marine coccolithophorid. In: Comparative biology of calcified tissue. Ann. N. Y. Acad. Sci. 109(1):49–64.

KINCAID, F. D., and B. T. SCHEER. 1962. Hormonal control of metabolism in crustaceans. IV. Regulation of tissue composition of *Hemigrapsus nudus* to intermolt cycle and sinus gland. Physiol. Zool. 25:372.

KRISHNAN, G. 1950. Sinus gland and tyrosinase activity in *Carcinus maenas*. Nature 165:364.

KYER, D. S. 1942. The influence of the sinus glands on gastrolith formation in the crayfish. Biol. Bull. 82:68.

LAFON, M. 1948. Nouvelles recherches biochimiques et physiologiques sur le squelette tégumentaire des crustacés. Bull. Inst. Oceanogr., Monaco 939:1.

MCWHINNIE, M. A. 1962. Gastrolith growth and calcium shifts in the freshwater crayfish, *Orconectes virilis*. Comp. Biochem. Physiol. 7:1.

MIYAWAKI, M., and N. SASAKI. 1961. A preliminary note on uptake of Ca^{45} by the hepatopancreas of crayfish, *Procambarus clarkii*. Kumamoto J. Sci., Ser. B, Sec. 2, 5:170.

NEEDHAM, A. E. 1946. Ecdysis and growth in Crustacea. Nature 158:667.

NETO, B. M. 1943. Calcium content of the carapace of *Uca maracoani* (Latreille). Anal. Soc. Biol. Pernambuco 4:31.

NUMANOI, H. 1939. Behavior of blood calcium in the formation of gastroliths in some decapod crustacea. Japan. J. Zool. 8:357.

ODUM, H. T. 1957. Biogeochemical deposition of strontium. Inst. Mar. Sci. 4(2):39.

PAUL, J. H. and J. S. SHARPE. 1916. Studies on calcium metabolism. I. The deposition of lime salts in the integument of decapod Crustacea. J. Physiol. 50:183.

PRENANT, M. 1927. Les formes mineralogiques du calcaire chez les êtres vivants, et le problème de leur déterminisme. Biol. Rev. Cambridge Phil. Soc. 2:365.

RIZZO, A. A., D. B. SCOTT, and H. A. BLADEN. Calcification of oral bacteria. In: Comparative biology of calcified tissue. Ann. N. Y. Acad. Sci. 109(1):14–22.

ROBERTSON, J. D. 1937. Some features of calcium metabolism of the shore crab (*Carcinus maenas* Pennant). Proc. Roy. Soc. London B, 124:162.

——— 1960. Ionic regulation in the crab *Carcinus maenas* (L.) in relation to the molt cycle. Comp. Biochem. Physiol. 1:183.

SATHER, B. T. 1966. Observations on the molt cycle and growth of the crab, *Podophthalmus vigil* (Fabricius). Crustacena 11(2):185–197.

SCHEER, B. T., and M. A. R. SCHEER. 1951. Blood sugar in spiny lobsters. Part I of the hormonal regulation of metabolism in crustaceans. Physiol. Comp. et Oecol. 2:198.

SCHÖNBORN, E. G. VON. 1912. Weitere Untersuchungen über den Stoffwechsel der Krustazeen. Zeitschr. f. Biol. 57:543.

SCUDAMORE, H. H. 1942. Hormonal regulation of molting and some related phenomena in the crayfish, *Cambarus immunia*. Anat. Record 84:514.

——— 1947. The influence of the sinus glands upon molting and associated changes in the crayfish. Physiol. Zool. 20:187.

SNEDECOR, G. W. 1959. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State College Press, Ames. 534 pp.

TRAVIS, D. F. 1954. The molting cycle in the spiny lobster, *Panulirus argus* Latreille. I. Molting and growth in laboratory maintained individuals. Biol. Bull. 107:433.

——— 1955a. Ibid. II. Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. Biol. Bull. 108:88.

———— 1955*b*. Ibid. III. Physiological changes which occur in the blood and urine during the normal molt cycle. Biol. Bull. 109:484.

———— 1957. Ibid. IV. Post-ecdysial changes in the hepatopancreas and integumental tissues. Biol. Bull. 113:451.

———— 1960. Matrix and mineral deposition in skeletal structures of the decapod Crustacea (Phylum Arthropoda), pp. 57–116. In: R. F. Sognnaes, ed., Calcification in Biological Systems. Am. Assoc. Adv. Science. Publ. No. 64.

———— 1963. Structural features of mineralization from tissue to macromolecular levels of organization in the decapod Crustacea. In: Comparative biology of calcified tissue. Ann. N. Y. Acad. Sci. 109(1):177–245.

URIST, M. R. 1962. The bone-body fluid continuum: calcium and phosphorus in the skeleton and blood of extinct and living vertebrates. Perspect. Biol. Med. 6:75.

VINOGRADOV, A. P. 1953. The Elementary Chemical Composition of Marine Organisms. Sears Found. Mar. Res., Memoir II, pp. 390–462.